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EXPERIMENT K-6-12

MORPHOMETRIC STUDIES OF ATRIAL GRANULES AND HEPATOCYTES

PART I. MORPHOMETRIC STUDY OF THE LIVER

PART II. THE ATRIAL GRANULAR ACCUMULATIONS

Principal Investigator:

**L. M. Kraft
NASA Ames Research Center
Moffett Field, CA. 94035**

Co-Investigators:

**L.C. Keil
NASA Ames Research Center
Moffett Field, California 94035**

**I.A. Popova
Institute of Biomedical Problems
Moscow, USSR**

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PART I: MORPHOMETRIC STUDY OF THE LIVER

L.M. Kraft

SUMMARY

The livers of flight, F, rats from the Cosmos 1887 mission were markedly paler and heavier than those of the synchronous, S, and vivarium, V, controls. In the F group, microscopic study revealed extensive hepatocytic intracytoplasmic vacuolization which was moderate in the S and minimal in the V groups. The vacuoles were not sudanophilic and therefore were regarded as glycogenic in origin. To obtain objective data concerning the extent of the vacuolization, livers were examined by computer assisted morphometry. Measurements of profile area and perimeter of the hepatocyte nuclei and vacuoles were evaluated according to stereological principles. Results indicated that the volume density of the nuclei was less in the F group than in the S ($p = <0.0002$) and V ($p = <0.001$) groups. Mean volume of individual nuclei did not differ. Volume density of the vacuoles was greater in the F than in the V group ($p = <0.02$) while their mean diameter was less ($p = <0.05$). To ascertain the relationship between increase in liver weight of the flight animals and the results of this study, an assumption was made that the specific gravity of the vacuolar contents was similar to the other extranuclear components of the hepatocyte. On that basis, calculations showed that the elevated vacuolar volume density in the flight group did not cause the increased liver weight in those animals, but that the non-nuclear, non-vacuolar parenchymal compartment did contribute significantly. Factors that may have played a causal role in liver weight and vacuolar compartment increases are discussed.

INTRODUCTION

When participation in the Cosmos 1887 mission was first proposed, a study of hepatic mitotic index was considered as an approach to elucidating the effects of space flight on developing organs in the juvenile animal. For this purpose, however, the rats from the Cosmos 1887 mission proved to be too old (105-111 days) at the time of necropsy. Even after extensive search, no mitoses were seen in any of the liver tissues studied.

Differences among the livers were noted, however. From gross observation it was evident that those of the flight group were markedly paler than the synchronous and vivarium control livers. Subsequent microscopic examination revealed that, whereas some cytoplasmic vacuoles were present in hepatocytes of all animals, the flight group was the most severely involved. As had been recorded by the recovery team in the USSR, the livers of that group were significantly heavier than those of the control groups (Grindeland, Vasques, et al.).

The goal of the present study was to characterize the vacuoles, to obtain data with which to evaluate the gross and microscopic differences, and, if possible, to explain the increased liver weight of the flight group from microscopic morphometric findings. In addition to the use of general histologic techniques, therefore, morphometry of the hepatocyte nuclei and intracytoplasmic vacuoles was undertaken using light microscopic computer assisted image analysis.

METHODS

Animals and Tissues.

A portion of the liver from each of five rats of flight, synchronous control and vivarium control groups was made available. At necropsy in the Soviet Union, the caudate lobe of each liver was cut into several small portions and immersed in cold (4°C) fixative consisting of 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. The tissues remained in the cold until they were further processed in Moscow by the American team members who replaced the fixative with a graded ethanol series up to a final concentration of 95%. During shipment to the United States and until histological preparations were made, the specimens remained at 4°C.

Tissue Preparation.

Because of the poor penetration by glutaraldehyde fixative after immersion, only the surface layer, about 1 mm thick, of each liver portion could be used for further processing. This well fixed layer was removed with a razor blade from most if not all of the poorly fixed core of each portion. Samples from each liver were then processed by one of the following methods:

1. for general oversight - standard dehydration through absolute ethanol to xylene, embedment in paraffin, sectioning at 4 μm , staining with hematoxylin and eosin;
2. for identification of vacuolar contents - embedment in Historesin (LKB Industries) directly from the 95% ethanol, sectioning at 1 μm , staining for neutral fat with oil red O in iso-propanol with Ehrlich's hematoxylin as counterstain, and mounting in glycerine-gelatin;
3. for morphometry of the nuclei - same as method 2., but staining only with Ehrlich's hematoxylin, and mounting in Permount;
4. for morphometry of the vacuoles - dehydration through absolute ethanol to n-butyl glycidyl ether (BGE), infiltration with BGE and a mixture of Quetol 651 (Ted Pella Inc) with hardener (nonenyl succinic anhydride [NSA]), embedment in the Quetol-NSA mixture, sectioning at 1 μm , and staining with 1% aqueous toluidine blue in 2% borax.

Morphometry and Stereology

Computer assisted morphometry was performed with the Zeiss IBAS (Kontron) image analysis system. The parameters measured were area and perimeter of the objects in question, and area of the reference fields.

Nuclei in 25 fields of view were measured in 4 μm Historesin-embedded sections stained with Ehrlich's hematoxylin. Care was taken to include representative fields from all lobular regions. These were selected at random but were included only if scanning indicated that fixation was adequate as evidenced by the appearance of the nuclei: noncrenulated, smooth perimeter (nuclear membrane); and readily identifiable nucleoli and/or structured nucleoplasm. As seen on the image monitor, the magnification was 1780x, representing an area of 7460 μm^2 .

Hepatic vascular, biliary, and lymphatic systems were excluded from the measurements. The proportion of the image occupied by parenchymal cells was calculated to be about 90 per cent overall, based on the sum of the tissue areas divided by the sum of the screen areas.

Vacuoles were measured for each animal in 10 random fields of the 1 μm Quetol embedded sections stained with toluidine blue. As seen on the image monitor, magnification was 4200x, and the total area of the field of view was 1194 μm^2 .

From the stored measuring data, stereological parameters were calculated by area analysis (Weibel et al. 1969). Pertinent to the present study were volume density (VV), numerical density (NV), and mean diameter (DQ) of objects. The formulas for these which include a correction factor for section thickness are.

$$VV = 2 \cdot U \cdot A / 3 \cdot \pi \cdot (t + 2/3 DQ) \cdot NA \cdot AT \text{ (x 100 for per cent volume),}$$

$$NV = NA / (t + 2/3 DQ) \cdot AT,$$

$$DQ = U / \pi \cdot NA,$$

where AT = sum of reference areas (μm^2),

A = sum of object profile areas in AT (μm^2),

U = sum of object perimeters in AT (μm),

NA = number of objects in AT.

t = section thickness, μm .

Because of the small group size, statistical evaluation was performed first by scanning the data with the Mann-Whitney non-parametric U-test using all combinations of group pairings. Results were confirmed using the two-sided t test. Significance of differences from the latter test are presented in the Results and accompanying Tables.

RESULTS

General Observations.

With the exception of the intracytoplasmic vacuolization, no abnormalities could be detected in the liver portions examined. Occasional binuclear cells and somewhat enlarged nuclei (polyploid?) were seen, but their incidence was regarded as normal.

Only very rare vacuoles in all groups were sudanophilic, staining with oil red O, and therefore containing triglycerides. The vast majority of the vacuoles appeared to be empty.

Morphometry: The nuclei.

Table 1 presents the results from analysis of the volume density, numerical density, and mean diameter of the hepatocyte nuclei. Volume density was significantly lower in the F than in the control groups ($F < S$, $p = <0.0002$; $F < V$, $p = <0.001$), but S and V did not differ from each other. All groups differed from each other with regard to numerical density of the nuclear compartment: $S < F$, $p = <0.001$; $F < V$, $p = <0.0005$; and $S < V$, $p = <0.02$. No differences in mean nuclear diameter were seen in any group pairings.

Morphometry: The intracytoplasmic vacuoles.

Table 2 presents the results of the vacuolar measurements. Here a greater volume density was seen only between the F and V groups ($p = <0.02$). Numerical density differed among all groups: $F > S$, $p = <0.001$; $F > V$, $p = <0.02$; and $S > V$, $p = <0.02$. Mean vacuolar diameter differed only between the F and V groups, where $F > V$, $p = <0.05$.

Additional calculations.

The data in Tables 1 and 2 were further calculated for each animal to determine the extent to which the nuclear and vacuolar compartments contributed to the increased weight of the flight animal livers.

Table 3 indicates such a possible relationship between liver weight and the nuclear and vacuolar volume densities, the estimation of which first required the assumption that the specific gravities of the vacuolar, nuclear, and residual parenchymal compartments were not significantly different, thus equating weight with volume. Second, calculations from the size of reference fields in relation to the image analyzer monitor field, indicated that the hepatic parenchymal cells comprised only about 90% of the total cell population in the regions examined. Therefore, liver weights were multiplied by 0.9 for the following calculations.

When the weight of the parenchyma (total liver weight \times 0.9) (Table 3, column A) was multiplied by the combined nuclear and vacuolar volume density values (column B) and the product (column C) subtracted from the parenchymal weight (column A), the residual parenchymal weight (column D) in the flight animals remained significantly greater than that of either of the controls ($F > S$, $p = <0.0005$; $F > V$, $p = <0.0005$).

Table 4 then shows that when the mean weight of the parenchymal compartment was subtracted from the total liver weight, the mean increase in parenchymal weight of the flight animals would have been 1.258 g, about 0.133 g less than the mean total liver weight increase, 1.395 g, in the flight group. Table 4 also indicates that values of the nuclear and vacuolar compartments did not contribute significantly to that liver weight increase, but that the remaining, or residual, parenchymal cell compartment did so, the two values, 1.258 and 1.284 g being almost equal.

DISCUSSION

Conditions likely to cause a pale liver such as that seen in the flight animals are fatty change and severe glycogenic infiltration. Histologic evidence for fatty change is lacking. The tissues prepared for determination of triglycerides had been treated only with aqueous solutions, the highest concentration of ethanol used was 95%, and the temperature during processing did not exceed 25°C. Had high concentrations of triglycerides been present in the parenchymal cells, many or most vacuoles would have been stained by the oil red O. Since only rare vacuoles were stained in all groups, severe glycogenic infiltration was regarded as the most likely cause of the pale appearance of the F group livers. Supporting this conclusion is the fact that glycogen would have been dissolved in the aqueous fluids used in processing, leaving empty spaces such as those seen in the preparations. Tissue fixation in an absolute ethanol/picric acid/formaldehyde solution would have retained glycogen within the vacuoles during processing, enabling specific staining.

Regarding morphometric results, hepatocytes of the F group manifested a lower nuclear to parenchymal volume ratio than did the control (S, V) groups, indicating a greater mean distance between the nuclei of the former. The larger vacuolar volume density in the F animals might then have accounted for at least some of the increased internuclear space. When, however, the vacuolar and nuclear volumes were taken into account, the residual parenchyma in the flight animals remained statistically greater than that of either control group and therefore contributed significantly to the increase in the liver weight in that group (Table 3). If there is a fallacy in these calculations, it would lie in the assumption that has been made, that is, that the specific gravity of the various cellular components is essentially the same for all of them. Using 90% as the parenchymal proportion of the liver may be valid only for the tissue examined, for Gates, et al. (1961) found that 30% of the human liver consists of parenchymal cells. Their study, however, encompassed the entire liver. In the present case, for example, no vessels larger than 30-40 μ m in diameter were

encountered, thereby enriching the specimens with parenchymal cells rather than with major vessels.

Regardless of the correctness of the assumption that has been made, there is little doubt that changes in the nuclear and vacuolar components were only minor contributors to the increased liver weight of the F animals, while the remainder of the hepatocytic cytoplasm contributed the major portion of the increase.

Among possible causes for that increase are hydropic change and/or markedly elevated intracellular glycogen in addition to that in the vacuoles. In this regard, the smaller mean diameter of the vacuoles in the flight group could have some implications as to genesis of their formation or, once they are formed, to their stability. Whether such knowledge would be helpful in determining the cause of their increased volume and numerical density will require additional investigation. Diet, feeding regimen, liver enzyme changes, hormonal effects, and "stress" have, among other factors, been studied by others, e.g. Babcock and Cardell, 1974; Cardell, 1971, in regard to liver changes, and it is well known that insulin and the glucocorticoids favor the accumulation of liver glycogen. Whether present data can be explained on the basis of such factors must await further study.

Alterations in other liver components and their contents (biliary system, lymphatic including Kupffer cells, vascular system) may also have taken place in the flight group. Although there is no evidence from the present histological preparations that would point to changes in those systems, they should be included in liver studies of future missions.

It is impossible to ascribe the present results to a particular leg of the Cosmos 1887 mission. Nevertheless, they may serve as background information on which interpretation of future flight data might be based.

CONCLUSIONS

1. The livers of flight animals on the Cosmos 1887 mission not only weighed more but also were paler in appearance than those of control groups.
2. Microscopically the principal difference between the flight animals and the controls was extensive intracytoplasmic (hepatocytic) vacuolization.
3. Based on lack of sudanophilia, which would have indicated the presence of triglycerides, the vacuoles were presumed to have contained glycogen.
4. Morphometric evaluation of nuclear volume densities in parenchymal cells demonstrated differences among the groups: $F < S$, $p = <0.0002$; $F < V$, $p = <0.001$.
5. Nuclear numerical density differed among all groups: $F < S$, $p = <0.001$; $F < V$, $p = <0.0005$; $S < V$, $p = <0.02$.
6. Mean diameter of the nuclei did not differ among the groups.
7. Volume density of the vacuoles differed only between the F and V groups: $F > V$, $p = <0.02$.
8. Numerical density of the vacuoles differed among all the groups: $F > S$, $p = <0.01$; $F > V$, $p = <0.02$; and $S > V$, $p = <0.02$.
9. Mean diameter of the vacuoles differed only between F and V groups: $F < V$, $p = <0.05$.

10. On the assumption that the specific gravity of the various parenchymal components were not significantly different from each other, calculations showed that the increased vacuolar compartment together with the nuclei did not contribute significantly to the increased liver weights in the flights, but that the residual (non-nuclear, non-vacuolar) parenchymal compartment constituted virtually all of that increase.

11. The cause of the alterations in the liver cannot be ascertained at this time, although such factors as "stress", diet, feeding regimen, hormonal and enzymatic changes, among others, need to be considered.

12. Although the results of this study cannot be ascribed to specific segments of the Cosmos 1887 mission, they may be useful as background information for future flights or ground based studies.

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TABLE 1
VOLUME DENSITY AND MEAN VOLUME OF HEPATOCYTE NUCLEI

| Group | Animal Number | N* | AT | VV | NV | DQ |
|-------|---------------|-------|------|-----------|-----------|-------|
| F | 06 | 191 | 1.84 | 0.0340 | 0.1026 | 9.147 |
| | 07 | 218 | 1.84 | 0.0365 | 0.1188 | 8.933 |
| | 08 | 208 | 1.85 | 0.0329 | 0.1145 | 8.732 |
| | 09 | 194 | 1.76 | 0.0344 | 0.1103 | 8.952 |
| | 10 | 254 | 1.85 | 0.0392 | 0.1413 | 8.580 |
| | Mean | 213.0 | 1.83 | 0.0354(a) | 0.1175(b) | 8.869 |
| | SD | | 0.04 | 0.0025 | 0.0146 | 0.218 |
| S | 06 | 264 | 1.83 | 0.0452 | 0.1448 | 8.933 |
| | 07 | 300 | 1.80 | 0.0554 | 0.1638 | 9.194 |
| | 08 | 287 | 1.74 | 0.0525 | 0.1639 | 9.077 |
| | 09 | 290 | 1.83 | 0.0517 | 0.1565 | 9.187 |
| | 10 | 281 | 1.75 | 0.0462 | 0.1643 | 8.649 |
| | Mean | 284.4 | 1.79 | 0.0502 | 0.1587 | 9.008 |
| | SD | | 0.04 | 0.0044 | 0.0084 | 0.227 |
| V | 06 | 291 | 1.81 | 0.0507 | 0.1611 | 8.960 |
| | 07 | 349 | 1.84 | 0.0500 | 0.1979 | 8.367 |
| | 08 | 305 | 1.83 | 0.0390 | 0.1791 | 7.940 |
| | 09 | 355 | 1.67 | 0.0612 | 0.2178 | 8.644 |
| | 10 | 372 | 1.71 | 0.0665 | 0.2200 | 8.847 |
| | Mean | 334.4 | 1.77 | 0.0535 | 0.1952 | 8.552 |
| | SD | | 0.08 | 0.0011 | 0.0253 | 0.410 |

* N, Number of nuclear profiles measured in reference area.

AT, sum of reference areas, $\mu\text{m}^2 \times 10^{-5}$.

VV, volume density.

NV, numerical density $\times 10^3$.

DQ, mean nuclear diameter, μm .

SD, standard deviation.

F, flight group.

S, synchronous control group.

V, vivarium control group.

(a), $F < S$, $p = <0.0002$; $F < V$, $p = <0.001$.

(b), $F < S$, $p = <0.001$; $F < V$, $p = <0.0005$; $S < V$, $p = <0.02$.

TABLE 2

VOLUME DENSITY AND MEAN VOLUME OF INTRACYTOPLASMIC VACUOLES

| Group | Animal Number | N* | VV | NV | DQ |
|-------|---------------|-------|-----------|----------|----------|
| F | 06 | 845 | 0.0172 | 42.75 | 0.983 |
| | 07 | 660 | 0.0109 | 35.22 | 0.853 |
| | 08 | 864 | 0.0183 | 44.36 | 0.946 |
| | 09 | 746 | 0.0149 | 38.33 | 0.945 |
| | 10 | 770 | 0.0173 | 39.07 | 0.975 |
| | Mean | 777 | 0.0157(a) | 39.94(b) | 0.940(c) |
| | SD | 82.0 | 0.0030 | 3.64 | 0.052 |
| S | 06 | 605 | 0.0098 | 31.71 | 0.896 |
| | 07 | 377 | 0.0144 | 18.09 | 1.117 |
| | 08 | 664 | 0.0160 | 33.16 | 1.015 |
| | 09 | 613 | 0.0181 | 29.62 | 1.099 |
| | 10 | 331 | 0.0073 | 16.95 | 0.952 |
| | Mean | 518 | 0.0131 | 25.91 | 1.016 |
| | SD | 152.3 | 0.0045 | 7.77 | 0.094 |
| V | 06 | 319 | 0.0080 | 15.76 | 1.042 |
| | 07 | 320 | 0.0129 | 15.36 | 1.117 |
| | 08 | 231 | 0.0066 | 11.22 | 1.085 |
| | 09 | 347 | 0.0122 | 16.63 | 1.121 |
| | 10 | 245 | 0.0046 | 12.74 | 0.915 |
| | Mean | 292.4 | 0.0089 | 14.34 | 1.056 |
| | SD | 51.2 | 0.0036 | 2.27 | 0.085 |

* N, number of vacuolar profiles measured in reference area.

Sum of reference areas for each animal was $1.194 \times 10^4 \mu\text{m}^2$.

VV, NV, DQ, SD, F, S, and V as for Table 1.

(a), $F > V$, $p = <0.02$.

(b), $F > S$, $p = <0.01$; $F > V$, $p = <0.02$; $S > V$, $p = <0.02$.

(c), $F < V$, $p = <0.05$.

TABLE 3

ESTIMATED RELATIONSHIP BETWEEN LIVER WEIGHT AND COMBINED NUCLEAR
AND VACUOLAR VOLUME DENSITY

| Group | Animal Number | A* | B | C | D |
|-------|------------------|-------|-------|-------|----------|
| F | 06 | 9.054 | 0.051 | 0.462 | 8.592 |
| | 07 | 9.216 | 0.048 | 0.442 | 8.774 |
| | 08 | 8.937 | 0.051 | 0.456 | 8.481 |
| | 09 | 8.658 | 0.049 | 0.424 | 8.234 |
| | 10 | 8.964 | 0.057 | 0.511 | 8.453 |
| | Mean | 8.966 | 0.051 | | 8.507(a) |
| | SD | 0.204 | 0.003 | | 0.198 |
| S | 06 | 7.506 | 0.055 | 0.413 | 7.093 |
| | 07 | 8.514 | 0.070 | 0.596 | 7.918 |
| | 08 | 7.866 | 0.068 | 0.535 | 7.331 |
| | 09 | 8.172 | 0.070 | 0.572 | 7.600 |
| | 10 | 7.830 | 0.054 | 0.423 | 7.407 |
| | Mean | 7.978 | 0.063 | | 7.470 |
| | SD | 0.381 | 0.008 | | 0.309 |
| V | 06 | 7.812 | 0.059 | 0.461 | 7.351 |
| | 07 | 8.028 | 0.063 | 0.506 | 7.522 |
| | 08 | 6.471 | 0.046 | 0.298 | 6.173 |
| | 09 | 7.308 | 0.074 | 0.541 | 6.767 |
| | 10 | 7.578 | 0.071 | 0.538 | 7.040 |
| | Mean | 7.439 | 0.062 | | 6.971 |
| | SD | 0.604 | 0.011 | | 0.532 |

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- *
 A, weight of liver parenchyma (liver weight x 0.9; see text).
 B, sum of nuclear and vacuolar volume densities; from Tables 1 and 2.
 C, product of A x B.
 D, A - C = residual liver parenchyma; see text for discussion of rationale for these calculations.
 F, S, and V as for Table 1.
 SD, standard deviation.
 (a), $F > S$, $p = <0.0005$, $F > V$, $p = <0.0005$.

TABLE 4

ESTIMATED CONTRIBUTION OF VACUOLES AND NUCLEI TO INCREASED
LIVER WEIGHT IN FLIGHT GROUP

| | F* | S | V | (S+V)/2 | F-[(S+V)/2] |
|----------------------------------|------------------|--------------|--------------|---------|-------------|
| Liver weight at necropsy (g), | 9.960 | 8.860 | 8.270 | 8.565 | 1.395(a) |
| Liver weight x 0.9, g | 8.966 | 7.978 | 7.439 | 7.708 | 1.258(b) |
| Contribution from: | | | | | |
| Nuclei | <u>0.317</u> (c) | <u>0.400</u> | <u>0.398</u> | 0.399 | -0.081 |
| Vacuoles | <u>0.141</u> | <u>0.105</u> | <u>0.066</u> | 0.086 | 0.055 |
| Residual parenchyma(c) | 8.508 | 7.473 | 6.975 | 7.224 | 1.284(d) |

* F, S, and V as for Table 1. All data in this Table are mean values.

(a), Difference between liver weight of flight and combined ground controls.

(b), Increase in weight of parenchymal tissue in flight group. Non-parenchymal tissue weight would be $1.395 - 1.258 = 0.133$ g. See text for rationale.

(c), The underlined values result from multiplication of the VV of nuclei or vacuoles (Tables 1 and 2) with the respective weight of the liver parenchyma for each group, F, S, or V.

(d), This value plus the nuclear and vacuolar portions add up to 1.115 g, the value at (b).

EXPERIMENT K-6-12

PART II: THE ATRIAL GRANULAR ACCUMULATIONS

L. Kraft and L. C. Keil

SUMMARY

Atrial myocytic intracytoplasmic granular accumulations composed of storage granules that are associated with the production of atrial natriuretic factor (ANF) were studied morphometrically in rats from the Cosmos 1887 mission. Those of the flight, F, group had a significantly greater volume density (VV) than of either the synchronous, S, ($F > S$, $p = <0.01$) or vivarium, V, ($F > V$, $p = <0.0005$) control groups, while the controls did not differ from each other in this respect. Number of granular accumulations per unit reference area (NR) was also increased in the flight animals ($F > S$, $p = <0.005$; $F > V$, $p = <0.0005$). Mean volume (VQ) of the individual granulated regions did not differ among the three groups. The increased VV in the flight group was therefore due to an increase in the number of granular regions rather than to their size. No differences were seen between right and left atria in any group for either VV, NR, or VQ. Possible reasons for the increase in the granular regions in the flight animal are discussed.

INTRODUCTION

Cytoplasmic granules are found in the atrial myocytes of all mammals. They are associated with the hormone, atrial natriuretic factor (ANF), which is involved in the regulation of blood pressure and volume and in the excretion of water, sodium, and potassium (de Bold, 1986; Ackermann and Irizawa, 1984). Because electrolyte imbalance and fluid shifts have been experienced by humans (and animals?) during space flight, it seemed appropriate to examine the ANF granulated regions in the atria of rats from the Cosmos 1887 flight and to ascertain, by means of morphometric and stereological methods (Weibel, 1969), if quantitative changes occurred in the flight animals. The method to accomplish this was based on the stereological procedure that had been employed by deBold (1975) for his morphometric and physiological studies of such granulated regions.

METHODS

Animals and Tissues

Five rats from each of three groups, the flight (F), the synchronous (S) and vivarium (V) controls, were studied.

At necropsy in the USSR the heart was cut transversely at the rostral pole of the ventricles, keeping the atria intact. The entire portion containing the atria was immediately immersed in cold, 4° C, fixative consisting of 3% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. The tissues remained in the cold until they were further processed in Moscow by the American team members who replaced the fixative with a graded ethanol series up to a final concentration of 95%. During shipment to the United States and until histological preparations were made, the specimens remained at 4° C.

Tissue Preparation

Although tissue penetration by glutaraldehyde is poor after immersion fixation, the thinness of most of the atrial wall is less than 1 mm and had therefore been well fixed for analysis.

The atria from each animal were processed separately in order to evaluate any differences between the right and left. From 95% ethanol, the tissues were dehydrated in absolute ethanol and xylene, embedded in paraffin (53° C), and sectioned at 5 μ m. Two sets of slides were made containing step sections taken at 50 μ m intervals through the entire extent of each atrium. One set of slides was stained with hematoxylin and eosin for general oversight purposes and the other with lead hematoxylin (deBold and Bencosme, 1975; deBold, 1979) for measurement of the granular accumulations. The tartrazine counterstain prescribed for the latter stain was omitted in order to enhance discrimination by the image analysis system.

Morphometry and Stereology

Computer assisted morphometry was performed with the Zeiss IBAS (Kontron) image analysis system. The parameters measured were area and perimeter of the granular regions and area of the reference fields. One field was chosen at random in each of 10 sections of each atrium. The sections selected were separated by at least 100 μ m (two steps), and the atrial regions in which the fields were measured comprised different levels of an atrium so that the entire atrium was surveyed. Thus, the total number of fields measured in this study was 300 (3 groups x 5 animals x 2 atria x 1 field x 10 sections). The magnification on the image monitor was 1780x and represented 7460 μ m² for each image.

Despite the absence of the tartrazine counterstain, interactive discrimination of the images was not satisfactory. Therefore, to make the measurements for area and perimeter, an editing function was used to outline the granular regions projected on the image monitor, and, because the reference area of the tissue did not always fill the monitored field, the same method was used to delineate the pertinent reference regions when necessary.

From the stored values for object (granular accumulation) area, object perimeter, and reference field area, the stereology program calculated volume density (VV) and mean volume (VQ) (μ m³) of the objects. Numerical density (NR) was calculated as number of objects per unit reference field area x 10³.

Statistical evaluation was performed using the two-sided t test with pairings among the three groups, F, S, and V.

RESULTS

Overview of the atria using the sections that had been stained with hematoxylin and eosin failed to detect any abnormalities.

Concerning the morphometric parameters, Table 1 indicates that there was no significant difference between right and left atria regardless of the group or parameter under consideration.

Table 2 presents the results for volume density, numerical density, and mean object volume for the three groups of animals, F, S, and V. The data constitute the mean of the right and left atrial measurements for each rat. Significant differences are seen only in volume density (F > S, p = <0.01; F > V, p = <0.0005) and numerical density (F > S, p = <0.005; F > V, p = <0.001). There were no differences among the groups in the size of the accumulations.

DISCUSSION

The absolute values for volume density obtained in this study differ from those in rats reported by de Bold (1978, 1979). In those studies, mean volume density (as per cent volume) of the granulated regions was as high as 3% in 6 week old rats and 4% in 10 week old rats of the same strain (Sprague Dawley, source not given). Factors which may account for this discrepancy include the sub-strain or source of the animals, various aspects of their husbandry such as the feed, feeding regimen, feed availability (food was withheld from the Cosmos rats for 22-24 hours before necropsy), general health status, age (the Cosmos animals were 15-16 weeks old), as well as other possible conditions related to the animals and/or their environment.

The method used in this study of outlining the granular region for the profile area data as opposed to the grid analysis used by de Bold could explain some of the difference as well. The precise boundary of a granular region is often indistinct and subject to some interpretation. In many instances a thin scattering of granules extends away from the main body of the accumulation. Including all of these scantily granulated regions in the measurement can lead to a large areal value and to a smaller value for the same region if they are omitted.

Regardless of the absolute values, however, if the methods of tissue preparation and the criteria for measuring the test and reference fields remain constant, results are likely to be meaningful as, in this study, they appear to be, for the differences between the flight and control groups are for the most part highly significant.

The lack of difference in the granularity between the right and left atria in the rats of the Cosmos 1887 mission is at odds with the statement of Cantin and Genest (1986) that the right contains from 2 - 2.5 times the number of granules as the left. Others do not mention whether their results refer to right, left, or both atria. Not only may different experimental conditions prevail in each study, but it must be emphasized that in this investigation granules were not counted, only their accumulated masses were measured.

The reasons for the increase in volume density of the granular regions in the flight group is not easy to explain nor is it possible to know which phase of the mission was responsible for the change, likely causes being reduced blood volume and/or body water content. The data show that the increase was due to an elevated number, rather than to an increased size, of the individual granular regions, but an exhaustive attempt at this time to interpret these findings in terms of their physiological importance would be speculative, especially because no chemical determinations of ANF were made. Nevertheless, the data might be regarded as background information for future ground based studies and space flights where biochemical determinations may be correlated with both microscopic and ultramicroscopic morphometry.

CONCLUSIONS

1. The granular regions in the atria of flight rats from the Cosmos 1887 mission and of synchronous and vivarium ground controls were studied morphometrically and evaluated using stereological principles.
2. Volume density and numerical density related to reference area were determined as was mean volume of the individual granular region.
3. No differences in those three parameters were seen between right and left atrium in any group.
4. The mean volume density of the granular regions was greater in the flight group than in the synchronous ($p = <0.01$) and vivarium control groups ($p = <0.0005$).

5. Number of granular regions in a unit reference area was greater in the flight animals than in the synchronous ($p = <0.005$) and vivarium groups ($p = <0.0005$).
6. Mean volume of the granular accumulations was not significantly different in any group pairing.
7. Increased volume density of the granular accumulations was due to increase in their numbers rather than to any increase in size.
8. By themselves the data do not explain physiological changes that may have occurred during the mission, although several factors may be responsible for the increase in the granular accumulations in the flight animals. Reduced blood volume and/or body water content seem most likely causes. The information obtained in this study may, however, be of value to future investigations of fluid and electrolyte balance in rodents during space flight missions.

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TABLE 1

VOLUME DENSITY, NUMERICAL DENSITY, MEAN VOLUME
OF RIGHT AND LEFT ATRIAL GRANULAR REGION

| Parameter | Group* | Left Atrium | | Right Atrium | | |
|-----------|--------|-------------|--------|--------------|--------|----|
| | | Mean | SD | Mean | SD | |
| VV | F | 1.741 | 0.408 | 1.552 | 0.260 | ns |
| | S | 1.271 | 0.164 | 1.211 | 0.256 | ns |
| | V | 1.109 | 0.179 | 1.139 | 0.217 | ns |
| NR | F | 1.439 | 0.244 | 1.277 | 0.342 | ns |
| | S | 1.033 | 0.641 | 1.044 | 0.220 | ns |
| | V | 0.880 | 0.133 | 0.916 | 0.190 | ns |
| VQ | F | 40.113 | 7.117 | 41.820 | 6.432 | ns |
| | S | 42.950 | 8.346 | 39.573 | 9.360 | ns |
| | V | 44.565 | 16.977 | 43.364 | 11.512 | ns |

* Number of animals in each group = 5.

F, flight group.

S, synchronous control group.

V, vivarium control group.

SD, standard deviation.

VV, volume density $\times 10^2$.

NR, numerical density, number of granular regions per $\mu\text{m}^2 \times 10^3$ of the reference field.

VQ, mean volume of granular accumulations, μm^3 .

ns, not significant, $p = >0.05$.

TABLE 2

VOLUME DENSITY, NUMERICAL DENSITY, AND MEAN VOLUME
OF GRANULAR REGIONS IN INDIVIDUAL ANIMALS

| Group | Animal Number | N* | A | AT | VV | NR | VQ |
|-------|------------------|-------|--------|-------|----------------------|----------------------|--------|
| F | 06 | 178** | 2059 | 1.455 | 1.416 | 1.224 | 38.636 |
| | 07 | 185 | 2498 | 1.421 | 1.764 | 1.309 | 45.712 |
| | 08 | 223 | 2557 | 1.444 | 1.769 | 1.551 | 38.189 |
| | 09 | 182 | 2551 | 1.445 | 1.496 | 1.259 | 41.783 |
| | 10 | 212 | 2599 | 1.456 | 1.787 | 1.456 | 40.662 |
| | Mean | 196 | 2374.8 | 1.444 | 1.646 ^(a) | 1.360 ^(b) | 40.996 |
| | SD | 20.2 | 247.0 | 0.014 | 0.176 | 0.139 | 3.018 |
| S | 06 | 160 | 1677 | 1.437 | 1.166 | 1.113 | 34.613 |
| | 07 | 131 | 1832 | 1.386 | 1.314 | 0.953 | 50.094 |
| | 08 | 156 | 2050 | 1.473 | 1.392 | 1.059 | 48.532 |
| | 09 | 132 | 2008 | 1.441 | 1.396 | 1.139 | 42.406 |
| | 10 | 138 | 1367 | 1.458 | 0.938 | 0.946 | 30.663 |
| | Mean | 143.4 | 1786.8 | 1.439 | 1.241 | 1.042 | 41.262 |
| | SD | 13.7 | 277.7 | 0.033 | 0.193 | 0.089 | 8.496 |
| V | 06 | 152 | 1514 | 1.549 | 0.975 | 1.006 | 31.636 |
| | 07 | 106 | 1708 | 1.406 | 1.223 | 0.735 | 58.966 |
| | 08 | 159 | 1735 | 1.517 | 1.153 | 1.047 | 34.379 |
| | 09 | 119 | 1567 | 1.446 | 1.085 | 0.812 | 46.542 |
| | 10 | 132 | 1805 | 1.532 | 1.185 | 0.863 | 48.298 |
| | Mean | 133.6 | 1665.8 | 1.490 | 1.124 | 0.893 | 43.965 |
| | SD | 22.1 | 121.2 | 0.061 | 0.098 | 0.131 | 11.117 |

* N, number of granular regions measured in reference area.

** All data are the means of the right and left atrial values.

A, profile area, μm^2

AT, reference area, $\mu\text{m}^2 \times 10^{-5}$.

VV, volume density $\times 10^2$.

NR, number of granular accumulations in reference area $\times 10^3$.

VQ, volume of granular accumulations, μm^3 .

SD, standard deviation.

F, S, and V as in Table 1.

(a), $F > S$, $p = <0.01$; $F > V$, $p = <0.0005$.

(b), $F > S$, $p = <0.005$; $F > V$, $p = <0.001$.